**EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION**
**ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES**
**Summary sheet of validation data for a diagnostic test**

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

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| **Laboratory contact details** | ILVO Institute for Agricultural and Fisheries Research<br>Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium |
| **Short description of the test** | Identification of Frankliniella occidentalis using COI barcoding |
| **Date, reference of the validation report** | 2024-06-03 - |
| **Validation process according to EPPO Standard PM7/98?** | no |
| **Is the lab accredited for this test?** | no |
| **Was the validated data generated in the framework of a project?** | no |
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| **Description of the test** | |
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| **Organism(s)** | Frankliniella occidentalis (FRANOC) |
| **Detection / identification** | identification |
| **Method(s)** | Molecular Sanger seq |
| **Method: Molecular Sanger seq** | |
| ***Reference of the test description*** | |
| **As or adapted from an EPPO diagnostic protocol** | yes |
| ***Other information*** | |
| **Other details on the test** | PM 7/129 COI loci |
| **Are the performance characteristics included in the EPPO diagnostic protocol?** | **no** |
| **Performance Criteria :** | |
| **Organism 1.:** | **Frankliniella occidentalis(FRANOC)** |
| **Analytical sensitivity** | |
| **What is smallest amount of target that can be detected reliably?** | PCR reaction not evaluated |
| **Diagnostic sensitivity** | |
| **Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98** | PCR reaction not evaluated |

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| **Analytical specificity - inclusivity** | |
| **Number of strains/populations of target organisms tested** | Phylogenetic analysis: 31 F. occidentalis sequences including 21 from EPPO-Q-bank and 10 from BOLD |
| **Specificity value** | 100% |
| **Analytical specificity - exclusivity** | |
| **Number of non-target organisms tested** | Phylogenetic analysis performed on 89 sequences from EPPO-Q-bank covering the following species: Frankliniella insularis (Fins) Frankliniella tenuicornis (Ften) Scirtothrips aurantii (Saur) Scirtothrips citri (Scit) Scirtothrips dorsalis (Sdor) Scirtothrips perseae (Sper) Thrips major (Tmaj) Thrips nigropilosus (Tnig) Thrips palmi (Tpal) Thrips tabaci (Ttab) and 170 sequences from BOLD Frankliniella tritici (Ftri) 10 Frankliniella panamensis (Fpan) 10 Frankliniella citripes (Fcit) 1 Frankliniella schultzei (Fsch) 10 Frankliniella gossypiana (Fgos) 10 Frankliniella insularis (Fins) 10 Frankliniella invasor (Finv) 10 Frankliniella intonsa (Fint) 8 Frankliniella gardeniae (Fgar) 10 Frankliniella fusca (Ffus) 10 Frankliniella andrei (Fand) 9 Frankliniella brunnea (Fbru) 4 Frankliniella minuta (Fmin) 8 Frankliniella unicolor (Funi) 3 Frankliniella tenuicornis (Ften) 10 Frankliniella borinquen (Fbor) 7 Frankliniella cephalica (Fecp) 10 Frankliniella bruneri (Fbru) 2 Frankliniella bispinosa (Fbis) 10 Frankliniella auripes (Faur) 2 Frankliniella rostrata (Fros) 1 Frankliniella parvula (Fpar) 1 Frankliniella fallaciosa (Ffal) 1 Frankliniella xanthaner (Fxan) 3 Frankliniella gemina (Fgem) 1 Frankliniella kelliae (Fkel) 4 Frankliniella australis (Faus) 1 Frankliniella longipennis (Flon) 1 Frankliniella regia (Freg) 2 Frankliniella aztecus (Fazt) 1 |
| **Specificity value** | 100% There is one COI sequence of F. borinquen (Fbor, red dot) that clusters with F. occidentalis. However, as 1) F. borinquen is very similar to F. occidentalis, 2) the sequence for this F. borinquen specimen was derived from NCBI, and 3) as other F. borinquen sequences cluster together at another location in the tree (other red dots), this is probably an incorrectly identified specimen. The sequence was thus not considered for the calculation of the inclusivity |
| **Test performance study** | |
| **Test performance study?** | no |
| **Other information** | |
| **Any other information considered useful** | For futher Wannes Dermauw : wannes.dermauw@ilvo.vlaanderen.be Jochem Bonte: jochem.bonte@ilvo.vlaanderen.be The wet lab part of the test (PCR) was not evaluated. --phylogenetic analysis details-- Alignment of 290 sequences using MAFFT with auto settings: % mafft --reorder --auto input IQ-tree details: AUTO detection model, 2000 UF bootstraps, 2000 SH-aLRT IQtree command: “iqtree -s |

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| | QBANK_BOLD_merged_Fo_MAFFT.fas -m TEST -bb 2000 -alrt 2000” layout phylogenetic tree: consensus tree of IQtree output was loaded in MEGA X; tree was midpoint rooted; green and red dots were added to Focc and Fbor, respectively; only bootstrap value > 95 are shown Species included in the tree: QBANK (all sequences with a “010#” or “BTQ###” suffix, and “EURL_1600162_Tnig” and “QBOL49_Ttab”; 110 sequences in total) Frankliniella insularis (Fins) Frankliniella occidentalis (Focc) Frankliniella tenuicornis (Ften) Scirtothrips aurantii (Saur) Scirtothrips citri (Scit) Scirtothrips dorsalis (Sdor) Scirtothrips perseae (Sper) Thrips major (Tmaj) Thrips nigropilosus (Tnig) Thrips palmi (Tpal) Thrips tabaci (Ttab)¬ BOLD (all other sequences; from each Frankliniella specimen determined at the species level in BOLD, a maximum of 10 (randomly selected) COI sequences was included.; 180 sequences in total; number of sequences for each species is shown) Frankliniella tritici (Ftri) 10 Frankliniella panamensis (Fpan) 10 Frankliniella citripes (Fcit) 1 Frankliniella schultzei (Fsch) 10 Frankliniella gossypiana (Fgos) 10 Frankliniella insularis (Fins) 10 Frankliniella invasor (Finv) 10 Frankliniella intonsa (Fint) 8 Frankliniella gardeniae (Fgar) 10 Frankliniella fusca (Ffus) 10 Frankliniella andrei (Fand) 9 Frankliniella brunnea (Fbru) 4 Frankliniella minuta (Fmin) 8 Frankliniella unicolor (Funi) 3 Frankliniella tenuicornis (Ften) 10 Frankliniella borinquen (Fbor) 7 Frankliniella cephalica (Fecp) 10 Frankliniella bruneri (Fbru) 2 Frankliniella bispinosa (Fbis) 10 Frankliniella auripes (Faur) 2 Frankliniella rostrata (Fros) 1 Frankliniella parvula (Fpar) 1 Frankliniella fallaciosa (Ffal) 1 Frankliniella xanthaner (Fxan) 3 Frankliniella gemina (Fgem) 1 Frankliniella kelliae (Fkel) 4 Frankliniella australis (Faus) 1 Frankliniella longipennis (Flon) 1 Frankliniella regia (Freg) 2 Frankliniella aztecus (Fazt) 1 Frankliniella occidentalis (Focc) 10 |
| | |
| The following complementary files are available online: | • Phylogenetic tree<br>• F_occidentalis_barcoding_alignment |

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